Oxytocin
Chemiluminescent Immunoassay Kit

User Manual

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Catalog # K3048-C1

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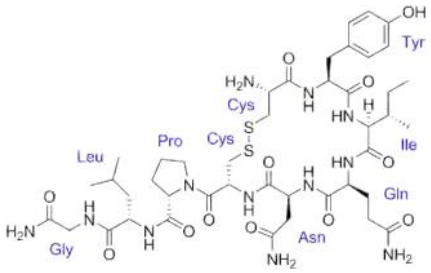
INTENDED USE

The B-Bridge Oxytocin Chemiluminescent Immunoassay kit is designed to quantitatively measure Oxytocin present in serum, EDTA and heparin plasma, clarified milk and tissue culture media samples. This assay is species independent.

BACKGROUND

Oxytocin is a neurohypophysial peptide which is produced in the paraventricular nuclei of the hypothalamus and stored in the posterior pituitary. The molecule consists of nine amino acids linked with a [1-6] disulfide bond and a semi- flexible carboxyamidated tail. A hormone once thought to be limited to female smooth muscle reproductive physiology and neurotransmitter, recent studies have begun to investigate Oxytocin's role in various behaviors, including orgasm, social recognition, pair bonding, anxiety, and maternal behaviors and is important in male reproductive physiology. Oxytocin and the related neurohypophysial peptide, Arg8-Vasopressin, maintain renal water and sodium balance.

Oxytocin



Highly conserved across species boundaries, Oxytocin-like neurohypophysial peptides are substituted primarily at residues 4 and/or 8. In the Oxytocin-like peptide, mesotocin, a common peptide found in some fishes, reptiles, birds, amphibians, marsupials and non-mammalian tetrapods, the leucine at residue 8 is substituted for isoleucine. Acting in classical endocrine fashion, Oxytocin elicits regulatory effects by binding specific cell surface receptors which in turn initiate a secondary intracelluar response cascade via a phosphoinositide signaling pathway.

ASSAY PRINCIPLE

The B-Bridge Oxytocin Chemiluminescent Immunoassay kit is designed to quantitatively measure Oxytocin present in serum, plasma, clarified milk and tissue culture media samples. This assay is species independent. Please read the complete kit insert before performing this assay.

1. Sample or standards are added to the well in a microtiter plate coated with an antibody to capture rabbit IgG.

2. Oxytocin-peroxidase conjugate is added to each well containing either standards or sample

3. The binding reaction is initiated by the addition of a polyclonal antibody to Oxytocin.

4. Incubate overnight at 4°C, wash plate, and add substrate to each well.

5. Substrate reacts with the bound Oxytocin-peroxidase conjugate to produce light. The intensity of the chemiluminescent signal is detected by a plate reader capable of measuring luminescence.

6. Calculate Oxytocin concentration from standard curve.

KIT COMPONENTS

Component:	Cat #	K3048-C1	K3048-C5
Coated White 96 Well Plates		1 plate	5 plates
Oxytocin Standard (50,000 pg/mL) in solution		125 uL	625 ul
Oxytocin Antibody		3 mL	13 mL
Oxytocin Conjugate		3 mL	13 mL
5X Assay Buffer		28 mL	55 mL
Extraction Solution		50 mL	250 mL
20X Wash Buffer		30 mL	125 mL
Substrate Solution A		6 mL	28 mL
Substrate Solution B		6 mL	28 mL
Plate Sealer		1 each	5 each

All components of this kit should be stored at 4°C until the expiration date of the kit.

MATERIALS REQUIRED BUT NOT SUPPLIED

- Distilled or deionized water
- Repeater pipet with disposable pipet tips capable of dispensing 25ul and 100ul
- A microplate shaker
- SpeedVac or other centrifugal evaporator to evaporate extracted samples.
- 96-well plate reader capable of reading glow chemiluminescence.

The number of RLUs obtained is dependant on the sensitivity and gain of the reader used. If you are unsure of how to properly configure your reader contact your plate reader manufacturer or carry out the following protocol:

Dilute 5 ul of the Oxytocin conjugate into 45 ul of deionized water. Pipet 5 ul of this dilution into an uncoated white well and add 100 ul of prepared CLIA substrate. This well will give you an intensity 2-3x the maximum binding for the assay. Adjust the gain or sensitivity so that your reader is giving close to the readers maximum signal.

To properly analyze the data software will be required for converting raw RLU readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. **Make sure all buffers used for samples are azide free.** Ensure that any plate washing system is rinsed well with deionized water prior to

using the supplied Wash Buffer .

In all cases, please consult your institution's safety procedures for working with hazardous chemicals.

REAGENT PREPARATION

Allow the kit reagents to thaw and come to room temperature for 30 minutes.

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine Oxytocin concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

Dilute 5X Assay Buffer 1:5 by adding one part Assay Buffer to four parts of deionized water. *Once diluted this is stable at 4°C for 3 months.*

Wash Buffer

Dilute 20X Wash Buffer 1:20 by adding one part of Wash Buffer to nineteen parts of deionized water. *Once diluted this is stable at 4°C for 3 months.*

Standard Preparation

1. Label test tubes #1 through #8.

2. Pipet 450 µL of 1X Assay Buffer into tube #1 and 300 µL of Assay Buffer into tubes #2 - #8.

3. Carefully add 50 µL of the Oxytocin stock solution to tube #1 and vortex completely.

   Note: The Oxytocin stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.

4. Take 200 µL of the solution in tube #1 and add it to tube #2 and vortex completely.

5. Repeat the serial dilutions for tubes #3 through #8. The concentration of Oxytocin in tubes 1 through 8 will be 5,000, 2,000, 800, 320, 128, 51.2, 20.48, and 8.192 pg/mL, respectively.

Use all Standards within 2 hours of preparation.

Reagent	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8
1X Assay Buffer	450 µl	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl
Oxytocin Stock	50 µl							
Standard 1		200 µl						
Standard 2			200 µl					
Standard 3				200 µl				
Standard 4					200 µl			
Standard 5						200 µl		
Standard 6							200 µl	
Standard 7								200 µl
Final Oxytocin Concentration (pg/mL)	5,000	2,000	800	320	128	51.2	20.48	8.192

Chemiluminescent Substrate
Mix one part Substrate Solution A with one part Substrate Solution B in a brown bottle. *Once diluted this is stable for one month when stored at 4°C.*

SAMPLE PREPARATION

This assay has been validated for serum, EDTA and heparin plasma, milk and tissue culture samples. Samples containing visible particulate should be centrifuged prior to use.

Oxytocin is identical across all species and we expect this kit may measure Oxytocin from sources other than human. Because of the cross reactivity to mesotocin this kit should also be able to measure mesotocin from birds, fish and amphibians. The end user should evaluate recoveries of Oxytocin in other samples being tested.

Serum and Plasma Samples
Serum and plasma samples should be extracted with the provided Extraction Solution or with a solid phase C18 column extraction protocol prior to running in the kit.

Protocol Using Extraction Solution:

- Mix 1 part sample with 1.5 parts of Extraction Solution.
- Vortex and then nutate at room temperature for 90 minutes.
- Centrifuge for 20 minutes at 4°C at 1660 x g.
- SpeedVac supernatant to dryness at 37°C.
- Reconstitute sample with 250 µL of Assay Buffer.

Milk Samples
Milk samples should be clarified by centrifuging at 10,000 x g for 15 minutes. Pierce the top fatty layer and collect the supernatant liquid. Repeat the centrifugation and collection two more times. The collected supernatant liquid must then be diluted ≥ 1:10 with the provided Assay Buffer before using in the assay.

The clarified milk sample, i.e., the supernatant liquid, can be stored at -20°C until needed.

Use all samples within 2 hour of preparation.

ASSAY PROTOCOL

1. The unused wells should be stored in the foil pouch with desiccant and stored at 4°C.
2. Pipet 100 µL of samples or standards into the appropriate number of wells in the plate.
3. Pipet 125 µL of 1X Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 100 µL of 1X Assay Buffer into wells to act as maximum binding wells (B0 or 0 pg/mL).
5. Add 25 µL of the Oxytocin Conjugate to each well using a repeater or multichannel pipet.
6. Add 25 µL of the Oxytocin Antibody to each well, except the NSB wells, using a repeater or multichannel pipet.
7. Shake the plate in a plate shaker at room temperature for 15 minutes to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and store at 4°C for 16 hours.

8. Aspirate the plate and wash each well 4 times with 300 µL 1X Wash Buffer. Tap the plate dry on clean absorbent towels.

9. Add 100 µL of mixed chemiluminescent Substrate to each well, using a repeater pipet.

10. Incubate the plate at room temperature for 5 minutes without shaking.

11. Read the luminescence generated from each well in a multimode or chemiluminescent plate reader using a 0.1 second read time per well. The chemiluminescent signal will decrease about 40% over 60 minutes.

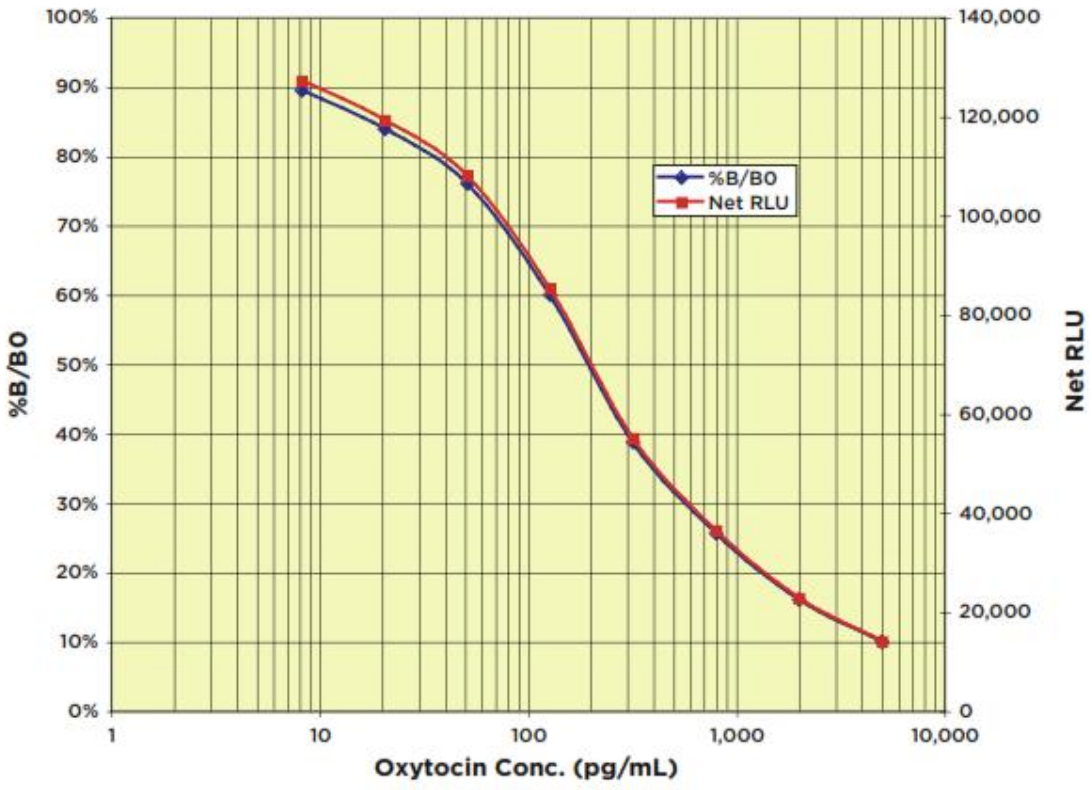
12. Use the plate reader's built-in 4PLC software capabilities to calculate Oxytocin concentrations for each sample.

CALCULATIONS

All luminometers read Relative Light Units (RLU). These RLU readings will vary with make or model of plate reader. Average the duplicate RLU readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean RLU's for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, and should be multiplied by the dilution factor to obtain neat sample values.

Conversion factor: 1 ng/mL of Oxytocin is equivalent to 0.993 nM.

TYPICAL STANDARD CURVE: EXAMPLE ONLY



Always run your own standard curve for calculations of results.

TYPICAL DATA: EXAMPLE ONLY

Sample	Mean RLU	Net RLU	% B/B0	Oxytocin Conc. (pg/mL)
NSB	6,600	0	-	-
Standard 1	20,735	14,135	9.94%	5,000
Standard 2	29,455	22,855	16.07%	2,000
Standard 3	43,100	36,500	25.67%	800
Standard 4	61,715	55,115	38.76%	320
Standard 5	91,965	85,365	60.04%	128
Standard 6	114,750	108,150	76.06%	51.2
Standard 7	123,370	119,320	83.92%	20.48
Standard 8	133,855	127,255	89.50%	8.192
B0	148,785	142,185	100%	0
Sample 1	32,505	25,905	18.22%	1,413.4
Sample 2	73,910	67,310	47.34%	229.1

Always run your own standard curve for calculations of results.